Oxygen Absorption of Methyl Esters of Fat Acids, and the Effect of Antioxidants

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KNOWLEDGE concerning the oxidation of the individual components of lard as well as their mixtures should be of value in disclosing the course of the development of oxidative rancidity. Furthermore the behavior of antioxidants with each component may help to reveal the nature of their protective action with lard.

Although the exact individual components of lard are not available, they may be represented, approximately, by methyl oleate, methyl stearate, and methyl linoleate. A number of workers have investigated the oxygen absorption of fat acids and esters (1-12), but no single investigation has included all the methyl esters of the fat acids of lard and mixtures of these methyl esters.

The usefulness of the Barcroft-Warburg apparatus in the study of the autoxidation of fats has been demonstrated (13,14) and it was our purpose to apply this convenient method to the methyl esters of fat acids representing the components of lard. Accordingly methyl oleate, methyl stearate, methyl linoleate, the distilled methyl esters of lard, and various mixtures of the individual methyl esters were prepared, and their oxygen absorption at 100° C. was determined. In addition, for general comparative purposes, measurements of the oxygen absorption of methyl linolenate were included.

A group of antioxidants was evaluated with methyl linoleate, methyl oleate, methyl stearate, and the distilled methyl esters of lard. The antioxidants were α-tocopherol, nordihydroguaiaretic acid (NDGA), propyl gallate, benzylhydroquinone, and synergistic combinations with citric acid, d-isoascorbyl palmitate, and lecithin.

As might be expected, methyl linolenate, methyl linoleate, the distilled methyl esters of lard, methyl oleate, and methyl stearate absorbed oxygen and developed peroxides, at relative rates in the order named. Mixtures of substrates absorbed oxygen at a rate which could be approximately predicted from the rate of oxygen absorption of the individual components. The order of effectiveness of the antioxidants varied somewhat with the different substrates but in each case the combinations of nordihydroguaiaretic acid and of propyl gallate with citric acid were the most powerful antioxidants.

Preparation of the Substrates

METHYL oleate was prepared from the methyl esters of lard by a series of low-temperature fractional crystallizations and fractional vacuum distillations (15). The final product had an iodine number of 85.5 (Wijs).

Methyl stearate was prepared from hydrogenated soybean oil fat acids by esterification with methyl alcohol; sulfuric acid was used as a catalyst. After fractional vacuum distillation and recrystallization the methyl stearate had a saponification equivalent of 300.4; a melting point of 39.0-39.4°; and an iodine number of zero (Wijs).

Methyl linoleate was prepared from the unsaturated fat acids of cottonseed oil (16) by the method of Rollett (17). Methyl linoleate distilled at 123-129° at .25 mm.; the saponification equivalent was 296.0; the iodine number (Wijs) was 172.4.

Methyl linolenate was prepared from the unsaturated fat acids of perilla oil (18) by the method of Rollett (17). The methyl linolenate distilled at 127-130° at .25 mm.; the iodine number was 260.5.

The distilled methyl esters of lard were prepared by methanolysis using .3% sodium. The methyl esters distilled at 114-133° at .12 mm.; the iodine number was 63.4; and the thiocyanogen number was 52.6. Assuming the absence of higher unsaturates, the esters contained 48.1% methyl oleate and 12.8% methyl linoleate.

Measurement of Oxygen Absorption

Oxygen absorption was measured in the Barcroft-Warburg apparatus in a manner similar to that described by Johnston and Frey (13). The flask was flushed with oxygen for 15 minutes at room temperature, and the system was then equilibrated at 100° for 10 minutes before pressure measurements were begun, zero time being selected as 5 minutes after equilibration had been started. The volume of the flask was about 80 ml.; the inner cup was 2.7 cm. high and had an inside diameter of 2.4 cm.

Modifications of the procedure of Johnston and Frey were that mercury was used as the manometer fluid, the size of the sample was 1 ml. (.87 g.), and a static rather than a shaking method was used. A 1-cm. layer of Brodie solution on top of the mercury levels in the manometer allowed the mercury to move more freely. These modifications made it convenient to permit a very slow oxygen absorption to proceed overnight or to record oxygen absorption, when considerable and rapid, without re-equilibration. The oxygen-absorption measurements were more readily duplicated when oxidation was rapid (for example, with methyl linolenate and methyl linoleate, which became slightly oxidized even during the equilibration period) than when oxidation was slow (for example, methyl oleate and methyl stearate). As an average figure, the difference between duplicate measurements was about 5%.

Peroxide values were determined only at the end of the experiment. As a general average, about 70% of the oxygen absorbed was present as peroxide, although this figure varied considerably in different experiments.

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Oxygen Absorption of Mixtures of Methyl Esters of Fat Acids

THE curves of Figure 1 and the data of Table 1 show the considerable effect of unsaturation, particularly dienes and trienes, in increasing the rate of oxygen absorption. The rate of oxidation of methyl linoleate, containing two double bonds, is much more than double the rate for methyl oleate. Previous workers have shown that the rate of oxygen absorption for linoleic acid is many times greater than that for oleic acid (1,2,6).

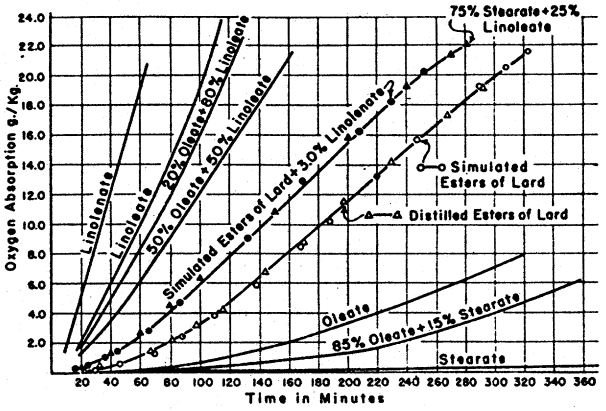

FIG. 1. Oxygen absorption of methyl esters of fat acids at 100° C.

The substrates are listed in Table 1 in the order of rapidity of oxygen absorption. It is apparent that the iodine number alone does not determine the rate of oxidation. A mixture of 50% methyl linoleate and 50% methyl stearate has about the same iodine number as methyl oleate, yet absorbed oxygen much more rapidly. Methyl stearate, which has an iodine number of zero, absorbed oxygen and formed peroxides. This was not unexpected, since stearic acid has been shown to oxidize under similar conditions (19).

TABLE 1.

Oxygen Absorption of Methyl Esters of Fat Acids at 100° C.

Substrate	Iodine Number	Oxygen Absorption	
% Methyl Esters		Minutes Required to Absorb 1g./kg.	Grams per Kilogram at 60 Minutes
100 Linolenate	260.4	7	19.1
100 Linoleate	172.4	11	10.2
90 Linoleate, 10 Stearate	155.2	13	10.0
80 Linoleate, 20 Oleate	155.0	15	8.4
50 Linoleate, 50 Oleate	129.0	17	6.6
50 Linoleate, 50 Stearate	86.2	18	5.0
25 Linoleate, 75 Stearate	43.1	35	2.6
20 Linoleate, 80 Oleate	103.0	36	2.9
100 Oleate	85.6	115	.2
85 Oleate, 15 Stearate	72.8	166	.2
57 Oleate, 43 Stearate	48.8	337	.1
100 Stearate	0	1250	.05

The curves of Figure 1 and the data of Table 2 show that the oxygen absorption of the simulated distilled methyl esters of lard (12.5% methyl linoleate, 50% methyl oleate, 37.5% methyl stearate) is about the same as that of the distilled methyl esters of lard (12.8% methyl linoleate, 48.1% methyl oleate). It is evident from the close correspondence of the two curves that any minor component, such as methyl arachidonate, that may be present in the distilled methyl esters of lard, does not greatly affect the overall oxygen absorption. The effect of .7% and of 3% methyl linolenate is shown in Table 2.

TABLE 2

Oxygen Absorption of the Methyl Esters of Lard at 100° C.

Substrate	Iodine No. of Substrate	Oxygen Absorption	
		Minutes Required to Absorb 1g./kg.	Grams per Kilogram at 60 Minutes
Simulated Distilled Me Esters of Lard + 3% Me Linolenate	70.0	34	2.6
Simulated Distilled Me Esters of Lard + .7% Me Linolenate	65.7	40	2.0
Distilled Me Esters of Lard [1]	63.4	55	1.2
Simulated Distilled Me Esters of Lard [2]	64.4	60	1.0

[1] Calculated composition, assuming the absence of higher unsaturates = 12.8% Me Linoleate, 48.1% Me Oleate, and 39.1% saturated compounds.
[2] 12.5% Me Linoleate, 50.0% Me Oleate, 37.5% Me Stearate.

The rate of oxidation of a mixture of methyl esters of fat acids is determined, as far as the limited accuracy of the present method can reveal, by the rate of oxidation of the individuals composing the mixture and the percentage of each present.

The present experiments suggest that in the development of oxidative rancidity each component in lard absorbs oxygen at a rate largely independent of the presence of others.

Effect of Antioxidants

The antioxidants were tested in a concentration of .01%. The mixture of substrate and antioxidant was prepared by pipetting the required amount of a dilute solution of the antioxidant in alcohol or petroleum ether into the inner cup of the reaction flask. The solvent was removed by passing a current of nitrogen through the flask. A weighed amount of substrate (.87 g. = 1 ml.) was then added to the inner cup. The flask was warmed, if necessary, to melt the substrate and then placed upon a mechanical shaker for about 20 minutes to accomplish the solution of the antioxidant. This method was convenient and apparently the antioxidant is fully incorporated since the synergistic action of the difficutly soluble citric acid is quite evident in the results. A method such as this was necessary to minimize the exposure of the more reactive substrates to oxidation.

The data on the stabilizing action of the antioxidants on three substrates are presented in Table 3. Figure 2 shows the oxygen absorption curves with methyl linoleate as the substrate. Similar curves were obtained with the other substrates.

For purposes of uniformity an oxygen absorption of 1 gram per kilogram, corresponding to a theoretical peroxide value of 31.3 millimols per kilogram, was selected as the end of the induction period. The point at which the absorption rate becomes rapidly accelerated is about 1 gram for several of the curves of Figure 2 but actually it is nearer 2 grams of oxygen per kilogram in the case of NDGA and propyl gallate and their synergistic combinations with citric acid. No attempt was made to correlate organoleptic observations with the selection of the end of the induction period in the present experiments.

Citric acid shows marked synergism with each phenol. This effect has been demonstrated so frequently (21) that perhaps it would be expected with any phenolic antioxidant. The synergistic action is

TABLE 3

Effect of Antioxidants and Synergists on the Oxygen Absorption of Methyl Esters of Fat Acids
Concentration, .01%. Temperature, 100° C.

Antioxidant	Me Linoleate		Me Oleate		Distilled Me Esters of Lard	
	Stability,[1] minutes	Order of Stability	Stability, hours	Order of Stability	Stability hours	Order of Stability
None	11	10	2	10	1	10
α-tocopherol	41	9	8.5	9	3.5	8
α-tocopherol + C.A.[2]	67	8	35.0	5	8.5	5
α-tocopherol + soya lecithin + d-isoascorbyl palmitate[3]	78	7	36.0	4	4.5	7
NDGA[4]	141	3	43.5	3	9.5	3
NDGA + C.A.	210	1	135.0	1	31.3	1
Propyl gallate	96	5	34.2	6	8.7	4
Propyl gallate + C.A.	158	2	101.0	2	25.4	2
Benzylhydroquinone	85	6	13.0	8	3.0	9
Benzylhydroquinone + C.A.	126	4	22.5	7	5.2	6

[1] Time required to absorb 1 gm. of oxygen per kg.
[2] Citric acid.
[3] Concentration, .02%.
[4] Nordihydroguaiaretic acid (20).

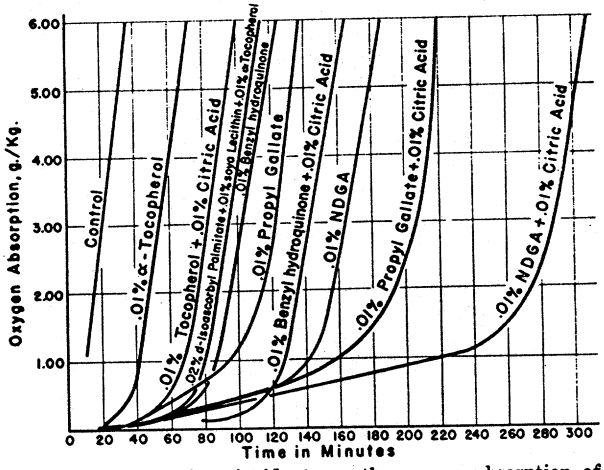

FIG. 2. Effect of antioxidants on the oxygen absorption of methyl linoleate.

greater with the methyl oleate than with the methyl linoleate substrate. The order in which the antioxidants arrange themselves varied somewhat with the different substrates, but the synergistic combinations of NDGA and propyl gallate with citric acid were the most effective in each case. and tocopherol and benzylhydroquinone alone were the least. NDGA, propyl gallate, and benzylhydroquinone, which have 4, 3, and 2 phenolic groups, respectively, were effective in the order of the number of their phenolic groups.

Data for the oxygen absorption of methyl stearate plus an antioxidant could not be duplicated with sufficient accuracy to warrant assigning a definite order of stability, and the results are not presented in Table 3. However, in a qualitative way NDGA and propyl gallate and their synergistic combinations with citric acid were again the most effective antioxidants. The other antioxidants had stabilizing action but to less degree. Methyl stearate alone had a peroxide value of 20 when 1 gram of oxygen per kilogram had been absorbed. The combinations of methyl stearate with the more effective antioxidants, however, had a zero peroxide even after 1 gram of oxygen per kilogram had been absorbed over a period of more than 100 hours at 100°

Benzylhydroquinone, propyl gallate, NDGA, and their synergistic combinations with citric acid were tested with lard in a similar manner. The relative order of effectiveness of the antioxidants was the same as with the methyl esters of lard.

Summary

The oxygen absorption of methyl linolenate, methyl linoleate, methyl oleate, methyl stearate, the distilled methyl esters of lard, and various mixtures of the four individual methyl esters were measured at 100° C. in the Barcroft-Warburg apparatus. Mixtures of methyl esters absorbed oxygen at a rate which could be approximately predicted from the rate of oxygen absorption of each component and the percentage of each present.

The antioxidants nordihydroguaiaretic acid (NDGA), propyl gallate, benzylhydroquinone, α-tocopherol, and their synergistic combinations with citric acid, d-isoascorbyl palmitate, and lecithin were tested with the substrates methyl linoleate, methyl oleate, methyl stearate, and the distilled methyl esters of lard. Citric acid showed marked synergism with each antioxidant. The two most effective were the combinations of citric acid with nordihydroguaiaretic acid and with propyl gallate.

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